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TITLE OF THE PROJECT: Chemical and Biological Attributes of Selected Periodontopathogens as Potential Indicators of Periodontal Disease

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I. SUMMARY

The outer sheaths from the three known serotypes of Treponema denticola have been prepared and were found to be homogeneous by chemical analysis, enzymatic assays, and electron microscopy. The relative molecular as well as the isoelectric points of the outer sheath polypeptides have been determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and two dimensional gel electrophoresis. The outer sheaths contained simple proteins as well as lipoproteins and glycoproteins. The polypeptide electrophoretic profiles of the 3 T. denticola serotypes a, b, and c were not identical.

Studies on the polypeptides of the outer sheath of T. denticola are of interest because of the paucity of data in this area and because these polypeptides may represent novel reagents for the early diagnosis of periodontal disease.

In another set of experiments designed to address the second aim of this investigation, the acid phosphatase of T. denticola serotype c has been found in the periplasmic space of this spirochete and has been purified 20-fold by DEAE-cellulose chromatography. This partially purified enzyme degraded a variety of phosphate containing substrates including p-nitrophenol phosphate, adenosine triphosphate, glucose-1-phosphate, and inorganic pyrophosphate. The addition of 40-100 μg/ml F^- induced a statistically significant reduction of the acid phosphatase. The useful radiolabeled, phosphorylated nutrients adenosine triphosphate and glucose-1-phosphate were readily taken up by the 3 serotypes of T. denticola, and this uptake was inhibited by such antiplaque agents as Cepacol, Viadent, Fluorigard and Plax. Other known potential periodontopathogens such as Fusobacterium nucleatum, Actinobacillus actinomycetemcoumitans and Bacteroides gingivalis bound statistically significantly smaller levels of the assay phosphorylated nutrients at low hydrogen ion concentrations found in the dental plaque.

The ability of T. denticola to take up and dephosphorylate nutrients at a greater rate than other potential periodontopathogens may allow the oral spirochetes to complete and thrive within the periodontal pockets with limited and nontransportable nutrients.

II. MAJOR RESULTS AND ACCOMPLISHMENTS

An acknowledged cause of periodontal disease is the dental plaque. The plaque bacteria include oral spirochetes which increase in samples from periodontal patients. THese plaque microorganisms produce enzymes, including phosphatase, metabolic products and cellular components which may be potentially harmful. Very little is known about the chemistry and biological properties of oral spirochetes.

Our investigation has three goals. The first goal is to determine the chemical and biological properties of the outer sheath of oral spirochetes. The second aim of our investigation is to examine the relative ability of oral spirochetes and other potential periodontopathogens for their relative ability to concentrate and utilize phosphorylated nutrients by sensitive radioisotopic assays. The third goal of our project is the determination of the potential role of phosphatase in the nutrition of oral spirochetes and in the possible pathophysiology of periodontitis. The overall aim of this project is to apply the information obtained from this study toward the early detection of periodontal disease, as well as toward a clear understanding of the pathophysiology and rational control of periodontal disease. During the first year of this investigation experiments have been conducted on the goals 1 and 2 of the research proposal.

Research Aim 1: Studied on the Chemical Properties of the Outer Sheath of T. denticola. One of our research goals is to determine the polypeptide profile of the outer sheath of oral spirochetes. Thus, the three known serotypes a, b, and c of T. denticola represented by ATCC strains 35405, 33521 and 35404 (or 33520) that have been originally isolated from periodontal patients, were grown anaerobically at 35°C for 5-10 days in a prereduced GM-1 medium prepared according to Blakemore and Canale-Parola (1). Cells from 15 liters of cultures of the assay treponemes in the late logarithmic, or early stationery phase of growth were removed by centrifugation at 12,000 g for 30 min, washed twice with deionized doubly distilled water, and the outer sheaths were removed by treatment for 15 min with 1.4 mM SDS (2). Dialysis of the outer sheaths against 20 mM $MgCl_2$ yielded the aggregated and the nonaggregated moieties of the outer sheaths (2). In contrast to previous isolations of spirochetal outer sheaths (2), in this study the outer sheaths were assayed for possible contamination of protoplasmic cylinder components using DNA, RNA and hexokinase as markers of cytoplasmic constituents, ATPase as cytoplasmic membrane marker, and muramic acid as well as ornithine as peptidoglycan markers (3-8). Furthermore, the morphology of the outer sheaths and the spirochetes from which the outer sheaths were removed was determined by electron microscopy (2).

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the solubilized outer sheath polypeptides was performed essentially as described by Laemmli (9). Detection of glycoproteins was performed by the periodic acid-schiff reagent assay (10). Lipoproteins were detected by placing the gel after electrophoresis in a solution containing Sudan Black. Controls included the omission the periodate treatment (10) for glycoproteins, or the removal of lipoproteins from the test samples by lipase. To suppress any possible proteolytic breakdown of the outer sheath proteins during

isolation, the serine-type protease inhibitor phenylmethyl sulfonyl fluoride was added to the distilled water used in the isolation of the outer sheath. Two dimensional electrophoresis was based on the method described by O'Farrell (11) with some minor modifications. Lipopolysaccharide assays for the outer sheaths involve the use of two methods. The first method consisted of pronase, or proteinase K digestion of the outer sheaths prior to SDS-PAGE, and silver staining the gels following periodate oxidation of polysaccharide (12). The second method was based on the one described by Kido et al., (13) and was essentially similar to the first method with the exception that hot phenol extraction for 10 min at 65°C was used to extract the lipopolysaccharide from the outer sheaths.

1a. Homogeneity of the Isolated outer Sheaths. Electron microscopy indicated that exposure of T. denticola to 1.4 mM SDS for 15 min removed the outer sheaths of the assay spirochetes without disturbing the typical protoplasmic cylinder. Similarly, biochemical analysis for DNA, RNA hexokinase, ATPase, muramic acid, or ornithine assays showed that both the aggregable and non-aggregable moieties of the outer sheath did not contain any detectable cytoplasmic contents, cell membrane, or peptidoglycan markers. The yield of the outer sheaths ranged between 4-7% of the cell dry weight, and was approximately equally divided between the aggregable and nonaggregable moieties. Thus, although the method used by Johnson et al., (2) for the extraction of the outer sheath of T. phagedenis biovar kazan 5 can be applied to the 3 known serotypes of T. denticola, the outer sheath yields are 3 times lower than those reported for T. phagedenis.

1b. Outer Sheath Polypeptide Characterization by SDS-PAGE. The nonaggregable moieties of the outer sheaths of T. denticola serotypes a, b, c showed 10, 8 and 12 Coomassie blue stained polypeptides respectively. The aggregable moieties upon SDS-PAGE revealed 22 Coomassie blue stained polypeptides for serotypes b and c and with a protein load of 100 μg. The majority of the polypeptides had relative molecular weights ranging between 14 and 97 kDa. Some faint bands had a relative molecular weight of 10 kDa. The electrophoretic polypeptides profiles of both moieties of the three serotypes showed many similarities. However, upon close examination they revealed slight variations. For example, the aggregable moiety of serotype a had a double band near the 66 kDa and single bands at 47, 45, and 28 kDa.

Detection of the outer sheath polypeptides was greatly improved by silver staining of the SDS-PAGE gels. A protein load of 2-15 μg resolved approximately 47 silver-stained polypeptides for both the aggregable and nonaggregable moieties of the outer sheaths of serotypes a, b, and c. The relative molecular weights ranged between 14 and 97 kDa. The electrophoretic profiles of the outer sheath moieties remain constant for each serotype. The electrophoretic polypeptide profiles of the aggregable and nonaggregable moieties shared many similarities, still they exhibited variation in the overall pattern, intensity, or location of the polypeptide stained zones. For example, the aggregable moiety of serotype a showed a well spaced ladder-like electrophoretic profile.

A comparison of the SDS-PAGE profiles of whole cell lysates with, or without the outer sheaths showed enhanced polypeptide banding or intensity

with relative molecular weights in the 110-10 kDa range. However, as in the case with whole cell lysates comigration of the numerous polypeptide zones did not result in precise delineation of polypeptide profile.

In some SDS-PAGE experiments, to prevent dissociation of the outer sheath proteins into polypeptides, the heat and mercaptoethanol treatment of samples was omitted. In these cases, the number of Coomassie blue stained bands was reduced by 50-75%. Thus, the nonaggregable moieties of serotypes a, b, c showed proteins with relative molecular weights of 110, 66, 53 and 42 kDa. The most pronounced protein gel band was that of 53 kDa for serotype a, 66 and 97 kDa for serotype b, and 97 kDa for serotype c. The aggregable moieties of the three serotypes showed Coomassie blue stained bands having relative molecular weights of 66, 53, 44, 43, 38, 32, and 28 kDa. Several proteins, which could not enter the gel could be seen at the top of the stocking gel with both the nonaggregable and aggregable moieties of the outer sheath of the 3 serotypes. Silver staining of the outer sheath aggregable and nonaggregable moieties revealed silver stained protein bands which had relative molecular weights of 110, 66, 53, 42, 38, 34, 28, 27, 26 and 24 kDa.

1c. Glycoproteins. An effort was made to assess the outer sheaths for the presence of glycoproteins. Therefore, following SDS-PAGE of 15 μg protein samples of the sheath proteins, the gels were processed for periodic acid-Schiff staining of glycopolypeptides (10). The experimental protocol revealed 13, 16 and 10 glycopolypeptides stained bands for the aggregable moiety of the serotypes a, b and c respectively. The nonaggregable moieties of the outer sheaths of serotypes a, b and c contained 6, 13 and 8 Schiff positive band respectively. The glycopolypeptides of the outer sheaths of the 3 serotypes were found to have relative molecular weights of 15-66 kDa. It is recognized that in the SDS-PAGE system, glycoprotein electrophoretic mobilities may not reflect reliable molecular weights (14). A known glycoprotein, horseradish peroxidase has been used as a positive control for the periodic acid Schiff reagent reaction. Omission of either the periodic acid treatment, or Schiff reagent yielded negative results.

These experiments indicated that there is variation in terms of number, location, or concentration of glycopolypeptides in the aggregable and non-aggregable moiety of each serotype, as well as between the 3 serotypes of T. denticola. For example, there appears to be a higher lever of a 29 kDa glycopolypeptide in the aggregable moiety of serotype b than the nonaggregable moiety. Similarly, the 29 kDa glycopolypeptide seems to be more intense in the aggregable moiety of serotype c and is absent in serotype a.

1d. Apo-lipopolypeptides. To determine the presence of apo-lipopolypeptides in the outer sheath of T. denticola, the resolved polypeptide components were stained with Sudan black B following SDS-PAGE. Other investigators (15) using Sudan B black for the identification of serum lipoproteins have demonstrated the usefulness of this stain.

The nonaggregable moieties of the outer sheaths of serotypes a, b and c upon SDS-PAGE and staining of the gels yielded 2, 7 and 2 Sudan black positive bands respectively. The 2 bands for serotype c appeared quite faint. The aggregable moieties of the outer sheaths of serotypes contained 4, 8 and 3

Sudan black positive bands for serotypes a, b and c respectively. Lipase treatment of the two outer sheath moieties prior to SDS-PAGE reduced the intensity, or sharpness of the Sudan black positive bands, and some bands no longer stained Sudan positive. With both the aggregable and nonaggregable moieties of the outer sheaths of the three serotypes the Sudan black positive bands appeared within the 15-66-kDa relative molecular weight range. The nonaggregable moiety of serotype b formed easily recognizable Sudan black positive bands at the 42 and 29 kDa relative molecular weight scale, while the same moiety of serotype a formed only a faint Sudan positive band at the 42 kDa area. A lipopolypeptide band that had in relative molecular weight of 50 kDa was present only in the aggregable moiety of the outer sheath of serotype a.

1e. Lipopolysaccharides (LPS). Owing to their unique content of certain carbohydrates and lipids, LPS have been a rich source of useful diagnostic markers (16). LPS may elicit a wide variety of pathophysiological effects during an infection (16). It was therefore pertinent to assay the outer sheath of T. denticola for the presence of LPS. LPS can be detected in biological material following the removal of proteins with proteolytic enzymes, such as proteinase k, or pronase, and silver staining of SDS-AGE gels (12, 17). Alternatively, the LPS may be removed from biological material with hot phenol (13). When such experiments were conducted, it was found that pronase, or proteinase k digestion of the nonaggregable moieties of serotypes a, b, and c yielded about 2, 6 and 5 LPS bands respectively, with relative molecular of 15 to 66 kDa. The 66 and 53 kDa bands were present in serotypes b and c but were absent in serotype a, while a band with approximate relative molecular weight of 45 kDa was present in serotype c, but not in serotypes a or b. The 66 and 53 kDa bands of serotype b were more prominent that those for serotype c. The 66 and 53 kDa LPS bands were found in the 3 serotypes of the aggregable moieties, but were very faint. Several bands with relative molecular weight o 10-15 kDa were very prominent in the aggregable moieties of the 3 serotypes, and they appeared very faint or absent in the nonaggregable portion of the outer sheaths of serotypes a, b and c.

To improve the resolution of the above described LPS bands hot phenol extraction of the LPS and SDS-PAGE experiments were conducted. The non-aggregable moiety of serotype b, had a distinct band with a relative molecular weight of 24 kDa that was absent from serotypes a or c. The 66 and 53 kDa bands were found in both the nonaggregable and aggregable moieties of serotypes a, b, c, as well as the Escherichia coli LPS, which was used as a control. It should be pointed out that the hot phenol LPS extraction procedure eliminated the 10 kDa band found in LPS preparations involving use of proteolytic enzymes.

1f. Two-dimensional protein electrophoretic studies. To obtain a more precise account of the number, the isoelectric points and relative molecular weights of the outer sheath polypeptides, two dimensional polypeptide analysis experiments were conducted (11, 18, 19). These experiments showed an excess of 100 silver stained polypeptides having isoelectric points of 4.2-7.0 and relative molecular weights of 14-110 kDa. The complexity of the polypeptides found both in the aggregable and nonaggregable moieties of serotypes a, b, c will require further experimentation to allow a meaningful description of

differences in the electrophoretic profiles of the outer sheath polypeptides.

Research Aim 2: Utilization of Phosphorylated Nutrients by Periodontopathogens. The second specific aim of our research plan is to assay potential periodontopathogens for their ability to concentrate radiolabeled, phosphorylated key nutrients following dephosphorylation by periplasmic phosphatases presumed to be present in the periplasmic space of T. denticola (20).

2a. A study on acid phosphatase of T. denticola. Further efforts were made to conclude prior investigations and provide additional evidence for the periplasmic location of acid phosphatase. A paper has been submitted and accepted for publication (21). This manuscript describes some of the properties of the potential periodontopathogen T. denticola serotype c (ATCC strain 33520). The highest enzyme activity was found in 87 h old cells. Two optimum pHs for enzyme activity were detected, one at pH 4.8 and another at pH 6.2. Divalent cations did not influence the acid phosphatase of T. denticola. The anion F^- added in the form of NaF and at a level greater than 20 μg/ml F^- diminished the activity of the acid phosphatase of intact cells of T. denticola. The addition of 10 μg/ml F^- as SnF_2 induced a statistically significant reduction of acid phosphatase activity.

The apparent K_m for the acid phosphatase was 7.3 mM with p-nitrophenyl phosphate as substrate. Fluoride appeared to be a noncompetitive inhibitor of the enzyme with an apparent K_i of 0.3 mM. Acid phosphate may be released partially in osmotic shock fluids. Also, 7-diazonium-1, 3-naphthalene disulfonate, which is incapable of penetrating the bacterial permeability barrier and is known to inactivate enzymes found in the bacterial periplasmic space, suppressed the activity of the acid phosphatase in intact cells of T. denticola.

The acid phosphatase could be released partially from the serotype c by the osmotic shock procedure (22) and a 20-fold purification of acid phosphatase has been achieved by DEAE-cellulose chromatography (23).

The enzyme degraded a variety of phosphate containing substrates including p-nitrophenol phosphate, adenosine triphosphate, glucose-1-phosphate, fructose-1, 6-diphosphate and inorganic pyrophosphate. The addition of 40-100 μg/ml F^- induced a statistically significant reduction of acid phosphatase.

2b. Studies on the Uptake of Selected Phosphorylated Nutrients by Periodontopathogens. The three serotypes a, b, and c of T. denticola were grown anaerobically in the GM-1 medium for 5-6 days. For the fast grown anaerobes Actinobacillus actinomycetemcomitans ATCC 29522, Bacteroides gingivalis ATCC 33277 and Fusobacterium nucleatum ATCC 25586 3 day old cultures were employed. The assay cells were collected by centrifugation and washed twice with deionized double distilled water. A homogeneous suspension was prepared by aspiration through a syringe with a 25-27 gauge needle. The suspensions were then adjusted to an optical density of 2.0 at 650 nm. The bacterial suspensions were incubated for 1 h in the anaerobic incubator to suppress the endogenous reserves of nutrients, and aliquots of the suspensions were used for the uptake experiments. The uptake was initiated by the addition of 0.5

ml of the bacterial suspension to 0.5 ml of the assay mixture containing 0.01 μCi [U-^{14}C] adenosine-5'-triphosphate, α-D-[U-^{14}C] glucose-1-phosphate, or D-[U-^{14}C] fructose-1, 6 diphosphate. Following incubation for varying time intervals, the cell pellets were washed twice with distilled water or acetate buffer. The cells were solubilized with 10% SDS and the radioactivity found in the solubilized cells was used to determine the uptake of radiolabeled, phosphorylated nutrients. Oral spirochetes heated at 65°C for 1 h were used as controls.

2c. Optimization of the Uptake of Phosphorylated Nutrients and Evaluation of Plaque Parameters Affecting Nutrient Uptake. These experiments were performed to systematically derive the operational conditions of phosphorylated nutrient uptake required for optimal response, as well as reproducibility, and to assess the dental plaque conditions that may influence nutrient uptake.

2d. Selection of Cell Concentration: Since the amount of phosphorylated nutrient accumulated by the bacterial cells may be small and only an adequate cell concentration can yield reliable, reproducible nutrient uptake, attempts were made to establish the optimal cell mass for the nutrient uptake studies. In general, washed cell suspensions of the assay cells at 0-5 mg, or more than 5 mg dry weight, were incubated anaerobically with sufficient levels of the radiolabeled, phosphorylated nutrient. Then, following incubation the uptake of the assay nutrient was determined by the general procedure. There was a linear relationship of the uptake of radiolabeled ATP or glucose-1-phosphate with cell concentrations in the range of 0.1-2.5 mg of cell dry weight. Above the 2-3 mg cell concentration level the uptake curve of the phosphorylated assay nutrients reached a plateau. Thus, in all of the subsequent uptake experiments 2-3 mg of cell dry weight were employed for the general assay system.

2e. Estimation of the End-point of Uptake. Meaningful and useful comparisons of uptake experiments of phosphorylated nutrients by test microorganisms can only be made with uptake systems that have attained equilibrium. Time course studies were conducted to determine the time interval required to attain the end point of the uptake of the assay nutrients. Thus, accumulation of the phosphorylated compounds by the test cells were allowed to proceed for varying time intervals and the time required for the uptake experiments to reach equilibrium were determined by the general assay procedure. The time course of the uptake of α-D-[U-^{14}C] glucose-1-PO_4, or [U-^{14}C] adenosine-5'-triphosphate was determined. The uptake of the radiolabeled assay nutrients reached a maximum value within 2-5 min and remained constant for the next 55 min. Therefore, for the ensuing uptake experiments a period of 30 min was used routinely. The percent uptake was nearly 3 times higher (27%) when the cells were washed with water, than with 0.2 M acetate buffer, pH 4.8. Hence water washed cells were used in all of the uptake experiments.

2f. Nutrient Uptake and Temperature. Conditions of growth in broth, saliva, dental plaque, and subgingival crevice where potential periodontopathogens may be found differ. Thus, the uptake of phosphorylated nutrients by the assay cells could be influenced by changes of temperature in the oral cavity or subgingival crevice. The accumulation of phosphorylated, radiolabeled ATP and glucose-1-phosphate was assessed by the usual general uptake procedure, but

under varying temperatures. At temperatures of 4-30°C the uptake ranged between 13-29%. At temperatures 50°C or higher than 50°C the assay cells took up 0-1% of the test nutrients.

2g. pH and Nutrient Uptake. The pH of the periodontal pocket varies with respect to pH. Therefore, it was pertinent to assess the uptake of radiolabeled assay nutrients under diverse hydrogen ion concentrations. It was found that uptake of [U-^{14}C] adenosine triphosphate was maximum at pH 4.8. α-D-[U-^{14}C] glucose-1-PO_4 uptake by the assay cells showed two pH maxima. The nutrient uptake maximum peaks for serotypes a and c occurred at pH 4.8 and 8.9, while for serotype b, which is thought to be more pathogenic than serotypes a, or c, occurred at pH 5.8 and 7.8

2h. Antiplaque Agents and Phosphorylated Nutrient Uptake. A number of antiplaque agents have been shown to decrease the number of subgingival motile bacteria, or the bleeding index score (24). The mechanism of action of these antiplaque remain uncertain, and it argues for further investigations in this area. To determine if inhibition of the uptake of the phosphorylated nutrients is occurring by interference with the dephosphorylating action of the phosphatases of the assay spirochetes, the following experiments were performed. Under optimal conditions established previously, cell suspensions of the assay organisms were incubated and processed as usual. However, the antiplaque agents were added to the assay system.

The five commercially available antiplaque agents known as Plax (Oral Research Laboratories), Cepacol (Lakeside Pharmaceuticals), Viadent (Viadent Co.), Listerine Antiseptic (Warner-Lampert Co.), and Fluorigard (Colgate-Palmolive Co.), at a dilution of 1:10 induced a 50-90, 50-70, 50-64, 50-56 and 8-41% inhibition respectively in the uptake of [U-^{14}C] adenosine triphosphate by the three serotypes. Stanous fluoride at a concentration of 10 μg/ml enhanced the uptake of radiolabeled ATP, or glucose-1-PO_4, indicating possible damage of the spirochetal membrane.

2c. Comparative Uptake of Phosphorylated Nutrients by Potential Periodontopathogens. A. actinomycetemcomitans, B. gingivalis and F. nucleatum, which have been implicated in the pathogenesis of periodontitis possess acid and alkaline phosphatase (20). Phosphatase could be involved in the dephosphorylation and subsequent uptake of various nutrients. Thus, the comparative accumulation of [U-^{14}C] adenosine triphosphate and α-D[U-^{14}C] glucose-1-phosphate by A. actinomycetemcomitans, B. gingivalis, F. nucleatum and T. denticola was determined by the general uptake assay procedure at a pH of 4.8 and 6.5. The uptake of phosphorylated ATP by A. actinomycetcomitans, B. gingivalis, F. nucleatum and T. denticola at pH 4.8 was 13, 1.5, 6.9 and 20.4% respectively. At a pH of 6.5 the percent uptake for the microorganisms indicated above was 22, 1.3, 6.6 and 6.2 respectively. The accumulation of radiolabeled, phosphorylated glucose-1-PO_4 at pH 4.8 was 4.2, 0.6, 0.7, 35.4% for A. actinomycetemcomitans, B. gingivalis, F. nucleatum and T. denticola respectively. At a pH of 6.5 the levels of accumulation of glucose-1-PO_4 by the assay cells changed to 16.7, 0.9, 4.3 and 4.9% respectively.

III. SIGNIFICANCE OF ACCOMPLISHMENTS IN REACHING OVERALL PROJECT GOALS

The overall goals of our investigation is to apply the information obtained from this study toward the early and rapid diagnosis of periodontal disease, as well as toward a sound understanding of the pathophysiology and rational control of periodontal disease.

The results presented in this report describe for the first time the number, the types, the relative molecular weights, and relative isoelectric points of the polypeptides found in homogeneous outer sheaths of the 3 known serotypes of T. denticola. Since the polypeptide profiles of the outer sheaths of the 3 serotypes that have been originally isolated from periodontal patients, are not identical, successful separation and use of the pertinent polypeptides could assist the taxonomy of oral spirochetes, or the early and rapid diagnosis of periodontal disease.

The presence of lipopolysaccharides, and glycoproteins in the outer sheath of T. denticola is another interesting finding brought out by this study. Virulence of oral spirochetes has been associated with the adherence of spirochetes to human cells, and glycoproteins appear to play a role in this attachment to human cells (25). Lipopolysaccharides, that are also known as endotoxin, have been shown to be a rich source of useful diagnostic markers, and they may elicit a wide variety of pathophysiological effects during an infection (16). For example, the following endotoxic effects can be observed clinically, or experimentally upon the administration of suitable levels of lipopolysaccharides: Fever, leukopenia, hypotension, impaired perfusion of essential organs and acidosis, activation of the third component of complement and the complement cascade, intravascular coagulation, and death.

The detection of lipopolysaccharides in the outer sheath of T. denticola suggests that lipopolysaccharides could be important in the development of periodontal disease by T. denticola, and it justifies further investigation on the biological attributes of the lipopolysaccharides of T. denticola. Furthermore, it lends support to the suspicion that structural components, especially the outer sheath which participates directly with the gingival cells and host defenses, may be involved in the pathogenesis of periodontitis (26).

During tissue destruction phosphorylated nutrients are released from oral tissues. There are fluctuations in the number and species of these plaque flora during health and dental disease. The factors governing these microbial changes in the dental plaque are not known. Nutrient uptake and utilization is thought to play a role in the observed bacterial ecology (26). This hypothesis is supported by the data obtained from this study. Furthermore, this study provides specific examples of phosphorylated nutrient accumulation by potential periodontopathogens. Such examples were lacking (27).

The metabolic versatility of T. denticola appears to contribute to its survival in the competitive environment of the gingival sulcus (27). The experiments on the uptake of radiolabeled, phosphorylated ATP and glucose-1-PO_4 tend to support the view that there are variations in the accumulation, or hydrolysis of useful phosphorylated nutrients by T. denticola, A. actino-

mycetemcomitans, B. gingivalis and F. nucleatum. These differences in nutrient uptake may contribute to the survival of T. denticola in the competitive environment of the gingival sulcus. The utilization of nucleotides such as ATP could involve prior dephosphorylation by periplasmic phosphatases. The periplasmic phosphatases act as scavaging enzymes, hydrolysing nontransportable, phosphorylated nutrients into components that can then be transported and utilized by microorganisms (23).

Limited information is available on the acid phosphatase of potential periodontopathogens. Initial studies in our laboratory indicate that T. denticola, A. actinomycetemcomitans, B. gingivalis and F. nucleatum possess acid phosphatase. However, while a 45-60 min incubation period was required to demonstrate this enzyme in T. denticola the other assay periodontopathogens required 180-240 min of incubation with p-nitrophenol phosphate (20) as substrate. Extension and confirmation of related work showed that useful substrates such ATP, glucose-1-phosphate, or other nutrients were hydrolyzed by a phosphatase that could be released from the periplasmic space of T. denticola, and be purified by DEAE column chromatography. Thus, the periplasmic acid phosphatase of T. denticola can act as a scavenging enzyme degrading nontransportable, phosphorylated useful nutrients that can then be taken up and utilized by this oral spirochete.

IV. PUBLICATIONS

(a) Full length paper accepted for publication

Norton-Hughes, C. A., and W. W. Yotis. 1990. A study of the acid phosphatase of Treponema denticola. Zbl. Bakt. 407BIS413.

(b) Published Abstracts

1. Yotis, W., Sharma, V. K., Hoerman, K., Keene, J. and Simonson, L. G. 1990. Outer Sheath polypeptides of Treponema denticola. J. Dent. Res. 69 (Sp. issue): 183 (Abstract No. 600).
2. Hughes, C. A. N., and Yotis, W. W. 1990. Isolation and partial characterization of acid phosphatase of Treponema denticola. J. Dent. Res. 69 (Sp. issue): 184 (Abstract No. 601).
3. Yotis, W., Gopalsami, C., Hoerman, K., Keene, J., and Simonson, L. 1990. Substitution of the anaerobic chamber with oxyrase for the growth of Treponema denticola. Absts. 90th Ann. Meeting of ASM, p. 213, Abstract No. I-87.
4. Sharma, V. K., Yotis, W. W., Keene, J., McNulty, J. and Simonson, L. 1990. Two dimensional polypeptide profile of the outer sheath of Treponema denticola. Absts. 90th Meeting ASM, p. 214, Abstract No. I-97.

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